

**Table 1. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors ( $\pm$  loxP)**

Plasmid combo	$\mu$ g DNA	Plaques/dish (293 cells)	(Totals)	Plaques/dish (293Cre4 cells)	(Totals)
<b>pCA36:pBHG10</b>	5:5	0, 0, 0, 0		0, 1, 2, 0	
	5:10	0, 0, 0, 1		1, 0, 0, 0	
	10:10	2, 0, 1, 1		1, 2, 0, 0	
			(5)		(7)
<b>pCA36:pBHGloxΔE1,3</b>	5:5	0, 0, 0, 1		0, 0, 0, 0	
	5:10	0, 0, 0, 1		0, 0, 0, 0	
	10:10	0, 0, 2, 1		0, 0, 0, 0	
			(5)		(0)
<b>pCA36lox:pBHG10</b>	5:5	1, 3, 1, 0		0, 1, 0, 1	
	5:10	0, 1, 0, 0		0, 0, 1, 2	
	10:10	0, 0, 0, 0		0, 1, 1, 0	
			(6)		(7)
<b>pCA36lox:pBHGloxΔE1,3</b>	5:5	1, 0, 0, 1		15, 14, 20, 20	
	5:10	0, 0, 0, 0		11, 15, 12, 16	
	10:10	0, 0, 1, 1		18, 9, 10, 8	
			(4)		(168)

**Table 2. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ Vectors ( $\pm$  loxP)**

Plasmid combo	$\mu$ g DNA	Plaques/dish (293 cells)	Plaques/dish (Totals)	Plaques/dish (293Cre4 cells)	Plaques/dish (Totals)
pCA36:pBHGloxΔE1,3	5:5	1, 1, 2, 6, 2, 3		1, 1, 2, 1, 2, 3	
			(15)		(10)
pCA36lox:pBHGloxΔE1,3	5:5	1, 2, 2, 2, 2, 1		41,44,41,41,44,31	
			(10)		(242)
pCA36loxΔ:pBHGloxΔE1,3	5:5	0, 0, 0, 0, 0, 0		41,36,55,34,24,40	
			(0)		(230)
<b>FG140</b>	1	72, 72		150, 115	

**Table 3 Efficiency of Ad vector rescue by cotransfection with pBHGloxΔE1,3 and various shuttle plasmids<sup>a</sup>**

Cell line	Shuttle plasmid	Plaques/dish	Average/dish
293	pCA36lox	6, 2, 3, 3, 5	3.8
	pCA36loxΔ	1, 4, 0, 0, 0	1.0
	pCA36loxΔCreR	2, 2, 4, 3, 2	2.6
	pCA36loxΔCreT	9, 4, 4, 7, 3	5.4
293Cre4	pCA36loxΔ	23, 28, 22, 28	25.3

<sup>a</sup> 5μg of all plasmids were used in cotransfections.

**Table 4. Efficiency of Ad vector rescue by cotransfection of 293 cells with pBHGloxΔE1,3 and shuttle plasmids encoding Cre<sup>a</sup>.**

Cell line	Shuttle plasmid	Plaques/dish	Average/dish
293	pCA36lox	2, 3, 1, 0, 1	1.4
	pCA36loxΔ	1, 0, 0, 0, 0	0.2
	pCA36loxΔCreT <sup>b</sup>	3, 1, 5, 2, 4	3.0
	pCA35loxΔCreITR <sup>b</sup>	21, 20, 42, 34, 40	31.4

<sup>a</sup>All cotransfections performed with 5 μg of the indicated shuttle plasmid and 5μg of pBHGloxΔE1,3

<sup>b</sup>Plasmids illustrated in figure 8c.

**Table 5. Efficiency of rescue of fibre and E4 genes into Ad by cotransfection with pFG173lox and pFG23lox<sup>a</sup>**

Plasmids	μg DNA	Number of plaques (average/dish)	
		293 cells	293Cre4 cells
pFG173lox <sup>b</sup> : pFG23dX1lox <sup>c</sup>	5:5	0, 0, 0, 0 (0)	33, 27, 39, 26 (31)
	2:2	0, 0, 0, 0 (0)	9, 15, 10, 9 (11)
pFG173 : pFG23dX1	5:5	0, 0, 0, 0 (0)	0, 0, 1 (0.3)
pFG140	1	95	93

<sup>a</sup>Cotransfections as diagrammed in figure 9

<sup>b</sup>Diagrammed in figure 9b

<sup>c</sup>Diagrammed in figure 10

**Table 6. Recombinant virus rescue following cotransfection of 293 cells with shuttle plasmids with or without a Cre expression cassette**

Plasmid	Number of plaques/dish (average/dish)
pCA36	1, 0, 0, 0 (0.3)
pCA36lox	1, 1, 1, 0 (0.8)
pCA36loxΔ	0, 0, 0, 0
pCA36loxΔCreT	2, 1, 2, 2 (1.8)
pFG140	40, 31 (35.5)

293 cells were cotransfected with 5 µg of pBHGloxΔE1,3 and 5 µg of the indicated shuttle plasmid or 1 µg of pFG140

**Table 7. Efficiency of Ad vector rescue by cotransfection of 293 cells with pBHG10 and shuttle plasmids with a single ITR or an ITR junction**

Plasmid	μg of DNA/60 mm	# of Plaques	Average
pCA35:pBHG10	2:2	2, 0, 0, 1, 0, 2	0.83
	5:5	2, 2, 2, 1	1.75
pCA35ITR:pBHG10	2:2	19, 11, 14, 12	14
	5:5	23, 23, 14, 17	19.25
pFG140	1	96, 106	101

**Table 8. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors ( $\pm$  lox,  $\pm$  ITR junction,  $\pm$  CRE)**

Shuttle Plasmid <sup>a</sup>	Plaques/dish (293 cells)	(Average)	Plaques/dish (293Cre4 cells)	(Average)
pCA36lox	0, 0, 0, 0	( 0 )	13, 15, 3, 13	(11)
pCA35loxITR	8, 13, 21, 19	(15)	111, 131, 100, 130	(113)
pCA36loxΔ	0, 0, 0, 0	( 0 )	10, 8, 9, 12	(10)
pCA35loxΔITR	0, 0, 0, 0	( 0 )	91, 127, 141, 118	(119)
pFG140 <sup>b</sup>		75		83

<sup>a</sup> All cotransfections 5 $\mu$ g shuttle plasmid + 5 $\mu$ g pBHGloxΔE1,3

<sup>b</sup> 1 $\mu$ g/dish

**Table 9. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors ( $\pm$  lox,  $\pm$  ITR junction,  $\pm$  CRE)**

Plasmids	$\mu$ g DNA /dish	Number of plaques /dish (average/dish)	
		293 cells	293Cre4 cells
pCA36:pBHGlox $\Delta$ E1,3	5:5	ND	0,3 (1.5)
pCA36lox:pBHGlox $\Delta$ E1,3	2:2	ND	9,3 (6)
	5:5	2,0,0,0 (0.5)	30,31,30,30 (30.25)
pCA35lox $\Delta$ CreITR:pBHGlox $\Delta$ E1,3	2:2	ND	71,60,56,79 (66.5)*
	5:5	36	100,96 (98)
pCA35lox $\Delta$ ITR:pBHGlox $\Delta$ E1,3	2:2	ND	55,64,75,63 (64.25)*
	5:5	0	120,113 (116.5)
pCA35loxITR:pBHGlox $\Delta$ E1,3	2:2	ND	53,54,61,66 (58.5)*
	5:5	ND	130,126 (128)
pFG140 (DC)	1	92	178
pFG140 (CE)	1	94	118

\* 5 plaques picked from each of these cotransfections and analyzed. All + for  $\beta$ -gal and all had predicted viral DNA structure

**Table 10. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors ( $\pm$  lox,  $\pm$  ITR junction,  $\pm$  CRE)**

Plasmids*	ugDNA/dish	Number of plaques/dish (average/dish)	
		293 cells (average)	293 Cre4 cells (average)
pCA36	5	1,0,0,0 (0.3)	1,0,0,0 (0.3)
pCA36lox	5	1,1,1,0 (0.8)	10,18,6,7 (10.3)
pCA36lox $\Delta$	5	0,0,0,0	6,4,3,0 (3.25)
pCA36lox $\Delta$ CreT	5	2,1,2,2 (1.8)	4,4,2, (3.3)
pCA35lox $\Delta$ CreITR	5	14,23,25,23 (21.3)	116,79,83,100 (94.5)
pCA35lox $\Delta$ ITR	5	0,0,0,0 (0)	65,62,64,51 (60.5)
pCA35loxITR	5	4,3,4,0 (2.8)	114,101,75,79 (92.25)
pFG140 (DC)	1	40,31 (35.5)	106,92 (99)
pFG140 (CE)	1	21,19 (20)	44,42 (43)

\*cotransfections with 5 $\mu$ g pBHGlox $\Delta$ E1,3 except for pFG140

**Table 11. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors ( $\pm$  lox,  $\pm$  ITR junction,  $\pm$  CRE)**

Genomic plasmid <sup>a</sup>	Shuttle plasmid <sup>a</sup>	293 cells	293Cre4 cells
pBHGloxΔE1,3	pCA36	2,3,1,2 (2)	3,3,3,1 (2.5)
	pCA36loxΔ	0,0,0,0 (0)	9,23,20,19 (17.8)
	pCA35loxITR	26,27,15,12 (20)	91,101,95,86 (93)
	pCA35loxΔCreITR	56,42,50,74 (55.5)	94,90,96,92 (93)
pBHGloxΔE1,3Cre	pCA36	1,1,0,0 (0.5)	2,3,2,0 (1.8)
	pCA36loxΔ	6,5,4,3 (4.5)	20,14,28,24 (21.5)
	pCA35loxITR	77,67,78,76 (74.5)	125,120,130,135 (128)
	pCA35loxΔCreITR	40,46,47,34 (41.8)	83,90,88,89 (87.5)
pBHGloxΔE1,3CreR	pCA36	0,0 (0)	ND <sup>b</sup>
	pCA36loxΔ	2,0 (1)	ND
	pCA35loxITR	39,29 (34)	ND
	pCA35loxΔCreITR	7,6 (6.6)	ND
pFG140		61,52 (56.5)	85,87 (86)

<sup>a</sup> Cotransfections with 5 µg each plasmid/dish except 1 µg/dish for pFG140

<sup>b</sup> Not done

**Table 12. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors ( $\pm$  lox,  $\pm$  ITR junction,  $\pm$  CRE)**

Plaques / dish (average / dish )				
Genomic plasmid	Shuttle plasmid	ug DNA /dish	293 cells	293Cre4 cells
pBHGloxΔE1,3	pCA36	5:5	2,2,1,0 (1.25)	2,1,1,1 (1.3)
	pCA36loxΔ	5:5	2,0,0,0 (0.5)	26,28,25,27 (26.5)
	pCA35loxITR	2:2 5:5	ND <sup>a</sup> 3,9,6,6 (6)	75,90 (82.5) TNTC <sup>b</sup>
	pCA35loxΔITR	2:2 5:5	ND 1,2,1,0 (1)	55,64 (59.5) TNTC
	pCA35loxΔCreITR	2:2 5:5	ND 33,28,35,31 (31.8)	61,64 (62.5) TNTC
pBHGloxΔE1,3 Cre	pCA36	5:5	2,2,1,0 (1.25)	4,1,1,0 (1.5)
	pCA36loxΔ	5:5	6,4,4,6 (5)	21,25,21,17 (21)
	pCA35loxITR	2:2 5:5	ND 57,49,45,54(51.3)	90,96 (93) TNTC
	pCA35loxΔITR	2:2 5:5	ND 39,45,39,46 (42.3)	75,87 (81) TNTC
	pCA35loxΔCreITR	2:2 5:5	ND 54,64,41,40(49.8)	117,103 (110) TNTC
pFG140		1	114,96 (105)	125,140 (132.5)

<sup>a</sup> Not done

<sup>b</sup> Too numerous to count

**Table 13. Cotransfections on 293 and 293Cre4 cells for rescue of LacZ vectors (CRE expressed from plasmids, by 293 cells, or both)**

Genomic plasmid	Shuttle plasmid	μgDNA/dish	Plaques/dish (average/dish)	
			293 cells	293Cre4 cells
pBHGloxΔE1,3	pCA35loxITR	5:5 2:2	3, 6, 9, 13 (8) 6, 4, 1, 3 (4)	TNTC <sup>a</sup> ( $\geq 109$ ) 65, 55, 64, 69 (63)
	pCA35loxΔITR	5:5 2:2	0, 0 (0) 1, 0 (0.5)	TNTC ( $\geq 117$ ) 49, 57, 47, 54 (52)
	pCA35loxΔCreITR	5:5 2:2	18, 21, 43 (27) 18, 12, 21, 24 (19)	TNTC ( $\geq 111$ ) 74, 61, 50, 49 (59)
pBHGloxΔE1,3Cre	pCA35loxITR	5:5 2:2	52, 66, 63, 57 (60) 48, 47, 32, 43 (43)	TNTC ( $\geq 116$ ) 72, 85, 69, 75 (72)
	pCA35loxΔITR	5:5 2:2	40, 36, 32, 63 (43) 48, 43, 52, 46 (47)	TNTC ( $\geq 122$ ) 93, 104, 106, 100 (101)
	pCA35loxΔCreITR	5:5 2:2	54, 56, 51 (54) 33, 37, 35, 19 (31)	TNTC ( $\geq 68$ ) 110, 94, 89, 83 (94)
pFG140		1	114	150

<sup>a</sup> Too numerous to count